

comprising

(A) an antisense polynucleotide which hybridizes with an RNA encoding the senescence-induced eIF-5A polynucleotide of the plant, and

(B) regulatory sequences operatively linked to the antisense polynucleotide such that the antisense polynucleotide is transcribed; and

(2) growing said plant, whereby said antisense polynucleotide is transcribed and binds to said RNA encoding the senescence-induced eIF-5A whereby expression of the senescence-induced eIF-5A polynucleotide is inhibited.

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### REMARKS

Claims 12, 42, 46, 50-51, 55, 58, 66, and 68-70 have been cancelled. Claims 13-17, 24-29, 32-36, 40-41, 47-49, 52-53, 56, and 67 have been amended. A copy of the amended claims to show the changes is included herewith and entitled "Version with Markings to Show Changes Made." Claims 37-39 remain unchanged. New claims 71-73 have been added. Applicants respectfully submit that the new claims fall properly within the elected group. No new matter has been added by the present claim amendments or claim additions. Accordingly, claims 13-17, 24-29, 32-36, 40-41, 47-49, 52-53, 56, 67 and 71-73 are pending in the present application.

### Claim Objections

Claims 12, 24-26, 46 and 50 have been objected for depending on a non-

elected claim. Claims 12-14, 28, 45, 50-51, 55, 56, 58, 66-67, and 69 have been objected to as they read on non-elected inventions. The claims have been amended to cancel the reference to non-elected claims and non-elected inventions. Accordingly, applicants respectfully request that the Examiner withdraw these claim objections.

**Claim Rejections under 35 U.S.C. §112, second paragraph**

Claims 12-17, 24-29, 32-42, 45-46, 48-53, 55-56, 58, and 66-70 have been rejected under 35 U.S.C. §112, first paragraph.

The Examiner has rejected various claims for the use of the term "low stringency conditions." The claim has been amended to recite "high stringency conditions," which chemical conditions have been defined in the specification at page 27, lines 23-27. Further, applicants respectfully assert that one skilled in the art would understand what is meant by "high stringency conditions."

The Examiner has rejected the simultaneous use of the terms "oligonucleotide or polynucleotide" in claim 14. Applicants note that the specification uses the terms oligonucleotide and polynucleotide interchangeably. Further, these terms are defined in the specification on page 8, second paragraph. But to further prosecution, the claims have been amended to recite a "polynucleotide."

The Examiner has rejected the use of the term "gene." The claims have been amended to delete this term and have used, as recommended by the Examiner, the term "polynucleotide" to denote nucleic acid molecules that

encode a polypeptide.

The Examiner has rejected the use of the term "about." The claims have been amended to remove this term.

The Examiner has rejected the use of the term "altered senescence" as unclear in claim 32. The claim has been amended to delete the term "altered" and replace it with the term "delayed." Support for this amendment is found on page 24, line 10.

The Examiner has rejected the use of various phrases using the term "increased" as it is a relative term and requires a comparative basis. The claims have been amended to include a comparative basis.

The Examiner has rejected claim 40 for stating that a plant is selected from a group consisting of fruit bearing plants, flowering plants, vegetables, etc. The claim has been amended to recited that the plant bears fruit, flowers, produces vegetables, is an agronomic crop plant or is a forest species plant.

The Examiner has rejected claim 45 alleging that the terms "age-related" and "environmental stress-related" are not defined and unclear. Regarding "environmental stress-related senescence," applicants respectfully disagree and assert that one skilled in the art would understand these terms as used in the claim and the specification. Further, applicants direct the Examiner to page 21, lines 13-15 where a few examples of environmental stresses are indicated. For example, the specification on page 7, lines 10-14 discusses some examples of environmental stress including dehydration, drought and chilling. Further, the term "age-related" is not unclear. One skilled in the art would understand this

term as referring to conditions surrounding the aging of a plant, its flowers or fruit. For example, on page 22, first paragraph, there is provided a discussion of flowers as they age. See also page 23, lines 16-20 for a discussion of effects of age on fruit, i.e. fruit softening and spoilage.

The remaining rejections have been rendered moot by claim amendments and claim cancellations. Accordingly, applicants respectfully request withdrawal of this ground of rejection.

#### **Claim Rejections under 35 U.S.C. §112, first paragraph**

Claims 12-17, 24-29, 32-42, 45-46, 48-53, 55-56, and 66-70 have been rejected under 35 U.S.C. §112, first paragraph. The claims have been amended or canceled and accordingly, have rendered this ground of rejection moot. However, in as much as the rejection pertains to the claims as amended, as well as the new claims, applicant provides the following explanations.

The Examiner asserts that the applicants have not reduced to practice the invention. The Examiner asserts that senescence is a complex and highly regulated process involving multiple pathways and that it is unpredictable what other proteins are required. The Examiner thus asserts that given the alleged lack of guidance and the unpredictability of what other proteins are required, excessive experimentation would be required to make and use the claimed invention. The Examiner further asserts that undue experimentation would be required by one skilled in the art to obtain an antisense molecule whose corresponding encoding DNA molecule would hybridize to SEQ ID NO:11 and

when expressed in plants would inhibit just senescence and not disrupt the normal processes required for plant growth.

Applicants respectfully disagree. Although senescence is a complex process, and although other proteins may be involved, the inventors are not claiming these other proteins, nor must a whole complex process be understood to claim a portion of the process that is understood and fully described in the specification.

The applicants direct the Examiner to the Examples in the specification. The disclosure in the specification, along with the Examples, enables one skilled in the art to obtain antisense polynucleotides of either DHS or senescence-induced eIF-5A that one could use to practice the various claimed methods, i.e. such as inhibiting the expression of endogenous senescence-induced eIF-5A polynucleotide in a plant.

First, the specification teaches that DHS and senescence-induced eIF-5A are involved in plant senescence. The specification lays out the relationship of DHS and eIF-5A, noting that DHS activates eIF-5A (page 3, lines 17-24.) Also, the specification notes that there is more than one isoform of eIF-5A and that the inventors have identified an isoform that is present in senescing cells -- what the inventors have termed "senescence-induced eIF-5A." See page 4, line 26-page 4, line 4 and page 5, lines 19-22. The specification teaches that using DHS nucleotide sequences in an antisense orientation to reduce the expression of DHS will reduce the levels of DHS protein and thus reduce or prevent the activation of eIF-5A. Alternatively, senescence-induced eIF-5A in an antisense

orientation can be used to reduce or inhibit the expression of the senescence-induced eIF-5A. In both alternatives, the amount of activated senescence eIF-5A is reduced. Examples 14 and 15 specifically show that by using DHS in antisense orientation to transform plants, the resulting plants and progeny displayed an increased biomass as well as delayed softening and spoilage of fruit, and less instance of physiological disease (blossom end rot, for example).

Thus, the specification teaches the relationship of DHS and senescence-induced eIF-5A and teaches how to use antisense polynucleotides to reduce their expression.

The specification also teaches and discloses (by way of the Examples) how the RNA coding for these proteins can be isolated (after induction with environmental and age related stress), how to create an antisense polynucleotide construct, and how to transform a plant cell with such construct. The specification has shows the results of transforming such a construct into a plant cell. See Example 14 and 15 (inhibiting gene expression resulting in delayed fruit spoilage (senescence), increased plant biomass, and less blossom end rot). Further, the specification even directs one skilled in the art to use the sequences provided in the specification to isolate RNAs encoding DHS and senescence-induced eIF-5A in other plants. See page 20, lines 11-15. One skilled in the art could apply the teachings of the specification and examples to these newly isolated DHS and senescence-induced eIF-5A polynucleotides in an antisense orientation to inhibit their expression in other plants.

Accordingly, applicants respectfully assert that they have indeed reduced

their invention to practice and have enabled one skilled in the art to practice the invention without undue experimentation. Applicant's respectfully remind the Examiner that some experimentation is allowed and Applicant's submit that since the specification provides working examples and the tools to apply these examples to other plants (i.e. the isolated sequences for DHS and senescence-induced eIF-5A), the experimentation would not be undue. Accordingly, applicants respectfully request withdrawal of this ground of rejection.

#### **Claim Rejections under 35 U.S.C. §101**

Claims 26, 27, 50-53, and 69-70 have been rejected under U.S.C. 101 as being directed to non-statutory subject matter. The claim amendments and cancellations have rendered this ground of rejection moot. Accordingly, applicants respectfully request withdrawal of this ground of rejection.

The Examiner has also rejected claim 56 as the claim is drawn to a method of inhibiting seed aging and hence is unpatentable since as one day passes to the next, the seed invariably becomes a day older. Claim 56 has been amended to clarify that the method is to reduce the effects of age on the seed.

#### **Claim Rejections under 35 U.S.C. §102**

The Examiner has rejected claims 26, 27, 50-53 and 69-70 under 35 U.S.C. §102(b) as being anticipated by Morrison et al. (U.S. patent 5,763,742) and claims 12-17, 2-27, 48-53, 66, and 69-70 as being anticipated by de Silva (U.S. patent 5,767,364). The claim amendments and cancellation have rendered

this ground of rejection moot. Further, Morrison et al. nor de Silva do not teach nor disclose the present invention. Morrison et al. is directed to a hybrid tomato produced by parental crossing. The de Silva patent relates to a xyloglucan-specific endo-(1-4)-beta-D-glucanase enzyme. Accordingly, applicants respectfully request withdrawal of this ground of rejection.

### CONCLUSION

Applicants submit that the claims are now in condition for allowance and earnestly request such action.

The Commissioner is authorized to charge any fees relevant to this filing to Deposit Acct No. 11-0600. The Examiner is invited to contact the undersigned at 202/220-4258 to discuss any matter in this application.

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Respectfully submitted,  
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**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

Claim 13 . (Amended) A vector for transformation of plant cells comprising the antisense polynucleotide of claim 14 and

[(a) antisense nucleotide sequences substantially complementary to (1) a corresponding portion of one strand of a DNA molecule encoding senescence-induced eIF-5A wherein the DNA molecule encoding senescence-induced eIF-5A hybridizes under low stringency conditions with SEQ ID NO:11, SEQ ID NO:13 and/or SEQ ID NO:15 or (2) a corresponding portion of an RNA sequence encoded by the DNA molecule encoding senescence-induced eIF-5A; and

(b)] regulatory sequences operatively linked to the antisense polynucleotide [nucleotide sequences] such that the polynucleotide is [nucleotide sequences are expressed] transcribed in a plant cell into which it is transformed.

Claim 14. (Amended) An antisense [oligonucleotide or] polynucleotide which hybridizes with RNA encoding senescence-induced eIF-5A, wherein said antisense polynucleotide [encoding an RNA molecule which is substantially complementary to (i) a corresponding portion of an RNA transcript of a plant senescence-induced deoxyhypusine synthase gene, wherein said plant gene] hybridizes under [low] high stringency conditions with [SEQ ID NO:1, SEQ ID NO:5 and/or SEQ ID NO:9 or (ii) a corresponding portion of an RNA transcript of a plant senescence-induced eIF-5A gene, wherein said plant gene hybridizes under low stringency conditions with] SEQ ID NO:11[, SEQ ID NO:13 and/or SEQ ID NO:15].

Claim 15. (Amended) The antisense [oligonucleotide or] polynucleotide according to claim 14 wherein the [oligonucleotide or] polynucleotide [comprises about] is from 6 [six] to [about] 100 nucleotides in length.

Claim 16. (Amended) The antisense [oligonucleotide or] polynucleotide according to claim 14 wherein the antisense [oligonucleotide or] polynucleotide [is substantially complementary] hybridizes to a [corresponding portion of the] 5'-non-coding region of [the RNA transcript] the RNA encoding senescence-induced eIF-5a.

Claim 17. (Amended) The antisense [oligonucleotide or] polynucleotide according to claim 14 wherein the antisense [oligonucleotide or] polynucleotide is [substantially complementary to a corresponding portion of the] hybridizes to a 3'-end of the RNA encoding senescence-induced eIF-5a [the RNA transcript].

Claim 24. (Amended) A bacterial cell transformed with the vector according to [any one of] claim[s 7, 12 or] 13.

Claim 25. (Amended) A transgenic plant cell [transformed with] comprising the vector according to [any one of] claim[s 7, 12 or] 13.

Claim 26. (Amended) A plant [and progeny thereof, wherein the plant is

generated] grown from [a] the plant cell [transformed with the vector according to any one of claims 7, 12 or 13] of claim 25.

Claim 27. (Amended) [A plant and] progeny [thereof, wherein] of the plant of claim 26, wherein the progeny comprise [is generated from a plant cell transformed with] the vector [according to any one] of claim[s 7, 12, or] 13.

Claim 28. (Amended) A method for inhibiting the expression of endogenous senescence-induced [deoxyhypusine synthase,] eIF-5A polynucleotide [or both] in a plant, said method comprising

(1) integrating into [the] a genome of at least one cell of the plant a vector comprising

(A) an antisense polynucleotide which hybridizes with RNA encoding the senescence-induced eIF-5A polynucleotide, wherein said antisense polynucleotide hybridizes under high stringency conditions with SEQ ID NO: 11 [sequences substantially complementary to

(i) a corresponding portion of one strand of a DNA molecule encoding the endogenous senescence-induced deoxyhypusine synthase, wherein the DNA molecule encoding the endogenous senescence-induced deoxyhypusine synthase hybridizes with SEQ ID NO:1, SEQ ID NO:5, and/or SEQ ID NO:9 or

(ii) a corresponding portion of an RNA sequence encoded by the endogenous senescence-induced deoxyhypusine synthase gene,

(iii) a corresponding portion of one strand of a DNA molecule encoding the endogenous senescence-induced eIF-5A, wherein the DNA molecule encoding the endogenous senescence-induced deoxyhypusine synthase hybridizes with SEQ ID NO:11, SEQ ID NO:13, and/or SEQ ID NO:15,

(iv) a corresponding portion of an RNA sequence encoded by the endogenous senescence-induced eIF-5A, or (v) a combination of (i) or (ii) and (iii) or (iv);] and

(B) regulatory sequences operatively linked to the antisense polynucleotide [sequences] such that the antisense polynucleotide [sequences are expressed] is transcribed; and

(2) growing said plant, whereby said antisense polynucleotide [sequences are] is transcribed and binds to said RNA encoding the senescence-induced eIF-5A [sequence,]

whereby expression of the [senescence-induced deoxyhypusine synthase gene,] senescence-induced eIF-5A polynucleotide [gene or both] is inhibited.

Claim 29. (Amended) The method according to claim 28 wherein the antisense polynucleotide [portion of the DNA or the portion of the RNA to which the antisense nucleotide sequence is substantially complementary comprises] hybridizes under high stringency conditions with a 5'-non-coding [or 3'-coding and/or non-coding sequences] region of an RNA transcript encoding senescence-induced eIF-5A.

Claim 32. (Amended) The method according to claim 28 wherein said inhibition results in [altered] delayed senescence of the plant as compared to senescence of a plant not having integrated into at least one cell of the plant the antisense polynucleotide.

Claim 33. (Amended) The method according to claim 28 wherein said inhibition results in increased resistance of said plant to environmental stress-induced and/or pathogen-induced senescence as compared to resistance to environmental stress and/or pathogen-induced senescence of a plant not having integrated into at least one cell of the plant the antisense polynucleotide.

Claim 34. (Amended) The method according to claim 28 wherein said inhibition results in increased biomass of said plant as compared to a biomass of a plant not having integrated into at least one cell of the plant the antisense polynucleotide.

Claim 35. (Amended) The method according to claim 28 wherein said inhibition results in delayed fruit softening and delayed fruit spoilage [in] of said plant as compared to fruit softening and fruit spoilage of a plant not having integrated into at least one cell of the plant the antisense polynucleotide.

Claim 36. The method according to claim 28 wherein said inhibition results in increased seed yield from said plant as compared to seed yield from a

plant not having integrated into at least one cell of the plant the antisense polynucleotide.

Claim 37. The method according to claim 28 wherein the regulatory sequences comprise a constitutive promoter active in the plant.

Claim 38. The method according to claim 28 wherein the regulatory sequences comprise a tissue specific promoter active in the plant.

Claim 39. The method according to claim 28 wherein the regulatory sequences comprise a senescence-induced promoter active in the plant.

Claim 40. (Amended) The method according to claim 28 wherein said plant bears fruit, flowers, produces vegetables, is an [is selected from the group consisting of fruit bearing plants, flowering plants, vegetables,] agronomic crop plant[s and] or is a forest species plant.

Claim 41. (Amended) The method according to claim 28 wherein the plant is a tomato plant.

Claim 45. (Amended) A method of [altering] delaying age-related senescence and/or environmental stress-related senescence in a plant, said method comprising

(1) integrating into [the] a genome of at least one cell of the plant a vector

comprising

(A) an antisense polynucleotide which hybridizes with RNA encoding a senescence-induced eIF-5A polynucleotide, wherein said antisense polynucleotide hybridizes under high stringency conditions with SEQ ID NO: 11 [sequences substantially complementary to

(i) a corresponding portion of one strand of a DNA molecule encoding the endogenous senescence-induced deoxyhypusine synthase, wherein the DNA molecule encoding the endogenous senescence-induced deoxyhypusine synthase hybridizes with SEQ ID NO:1, SEQ ID NO: 5 and/or SEQ ID NO:9 or

(ii) at least a portion of an RNA sequence encoded by the endogenous senescence-induced deoxyhypusine synthase gene,

(iii) a corresponding portion of one strand of a DNA molecule encoding the endogenous senescence-induced eIF-5A gene, wherein the DNA molecule encoding the endogenous senescence-induced eIF-5A hybridizes with SEQ ID NO:11, SEQ ID NO:13 and/or SEQ ID NO:15,

(iv) a corresponding portion of an RNA sequence encoded by the endogenous senescence-induced eIF-5A gene, or

(v) a combination of (i) or (ii) and (iii) or (iv)];

(B) regulatory sequences operatively linked to the antisense polynucleotide [sequences] such that the antisense polynucleotide [sequences are expressed] is transcribed; and

(2) growing said plant, whereby said antisense polynucleotide [sequences

are] is transcribed and binds to said RNA encoding senescence-induced eIF-5A [sequence], whereby expression of [said senescence-induced deoxyhypusine synthase gene,] the senescence-induced eIF-5A polynucleotide [gene or both] is inhibited[.]; and

(3) whereby age-related and/or environmental stress-related senescence in the plant is delayed.

Claim 48. (Amended) A plasmid comprising a replication system functional in a prokaryotic host and an antisense [oligonucleotide or] polynucleotide according to claim 14.

Claim 49. (Amended) A plasmid comprising a replication system functional in *Agrobacterium* and an antisense [oligonucleotide or] polynucleotide according to claim 14.

Claim 52. (Amended) The plant of claim 26 [and progeny according to claim 51] wherein the plant is a tomato plant.

Claim 53. (Amended) The plant of claim 26 [and progeny according to claim 52] wherein the plant is a flowering plant.

Claim 56. (Amended) A method of [inhibiting seed aging] reducing effects of aging on seed, said method comprising



(1) integrating into [the] a genome of at least one cell of a plant a vector comprising

(A) an antisense polynucleotide which hybridizes with RNA encoding senescence-induced eIF-5A polynucleotide, wherein said antisense polynucleotide hybridizes under high stringency conditions with SEQ ID NO:11  
[sequences substantially complementary to

(i) a corresponding portion of one strand of a DNA molecule encoding an endogenous aging-induced deoxyhypusine synthase, wherein DNA encoding said endogenous aging-induced deoxyhypusine synthase hybridizes with SEQ ID NO:1, SEQ ID NO:5 and/or SEQ ID NO:9 or

(ii) a corresponding portion of a substantially complementary RNA sequence transcribed from a DNA molecule encoding an endogenous senescence-induced deoxyhypusine synthase gene,

(iii) a corresponding portion of one strand of a DNA molecule encoding an endogenous aging-induced eIF-5A gene, wherein DNA encoding said endogenous aging-induced eIF-5A hybridizes with SEQ ID NO:11, SEQ ID NO:13 and/or SEQ ID NO:15,

(iv) a corresponding portion of a substantially complementary RNA sequence transcribed from a DNA molecule encoding an endogenous senescence-induced eIF-5A gene; or

(v) a combination of (i) or (ii) and (iii) or (iv);] and

(B) regulatory sequences operatively linked to the antisense polynucleotide sequences; and

(2) growing said plant, whereby said antisense polynucleotide [sequences are] is transcribed and binds to said [substantially complementary] RNA encoding senescence-induced eIF-5A [sequence and] whereby expression of [said aging-induced deoxyhypusine synthase gene,] the senescence-induced eIF-5A polynucleotide [gene or both] is inhibited[.];

(3) allowing said plant to produce seed; and

(4) whereby the effects of aging on the seed is reduced.

Claim 67. (Amended) A method of increasing resistance to [physiological disease] blossom end rot in a tomato plant, said method comprising

(1) integrating into [the] a genome of at least one cell of the plant a vector comprising

(A) an antisense polynucleotide which hybridizes with RNA encoding senescence-induced eIF-5A polynucleotide, said antisense polynucleotide hybridizes under high stringency conditions with SEQ ID NO: 11 [sequences substantially complementary to

(i) a corresponding portion of one strand of a DNA molecule encoding the 3' end of endogenous senescence-induced deoxyhypusine synthase, wherein the DNA molecule encoding the endogenous senescence-induced deoxyhypusine synthase hybridizes with SEQ ID NO:31 or

(ii) at least a portion of an RNA sequence encoded by the 3' end of the endogenous senescence-induced deoxyhypusine synthase gene,

(iii) a corresponding portion of one strand of a DNA molecule

encoding the endogenous senescence-induced eIF-5A gene, wherein the DNA molecule encoding the endogenous senescence-induced eIF-5A hybridizes with SEQ ID NO:11, SEQ ID NO:13 and/or SEQ ID NO:15,

(iv) a corresponding portion of an RNA sequence encoded by the endogenous senescence-induced eIF-5A gene, or

(v) a combination of (i) or (ii) and (iii) or (iv);]

(B) regulatory sequences operatively linked to the antisense polynucleotide [sequences] such that the antisense polynucleotide [sequences are expressed] is transcribed; and

(2) growing said plant, whereby said antisense polynucleotide [sequences are] is transcribed and binds to said RNA encoding the senescence-induced eIF-5A polynucleotide [sequence,] whereby expression of said [senescence-induced deoxyhypusine synthase gene,] senescence-induced eIF-5A polynucleotide [gene or both] is inhibited, and

(3) whereby the resistance to blossom end rot in the tomato plant is increased as compared to resistance to blossom end rot in a tomato plant not having said antisense polynucleotide integrated into the genome of at least one cell of the plant.

Claim 71. (new) The method according to claim 28 wherein the antisense polynucleotide hybridizes under high stringency conditions with a 3'-coding region of an RNA transcript encoding senescence-induced eIF-5A.

Claim 72. (new) A method of increasing resistance to disease in a plant, said method comprising

(1) integrating into a genome of at least one cell of the plant a vector comprising

(A) an antisense polynucleotide which hybridizes with RNA encoding senescence-induced eIF-5A polynucleotide, said antisense polynucleotide hybridizes under high stringency conditions with SEQ ID NO: 11

(B) regulatory sequences operatively linked to the antisense polynucleotide such that the antisense polynucleotide is transcribed; and

(2) growing said plant, whereby said antisense polynucleotide is transcribed and binds to said RNA encoding the senescence-induced eIF-5A polynucleotide whereby expression of said senescence-induced eIF-5A polynucleotide is inhibited, and

(3) whereby the resistance to disease in the plant is increased as compared to the resistance to disease in a plant not having said antisense polynucleotide integrated into the genome of at least one cell of the plant.

Claim 73. (New) A method for inhibiting the expression of endogenous senescence-induced eIF-5A polynucleotide in a plant, said method comprising

(1) integrating into a genome of at least one cell of the plant a vector comprising

(A) an antisense polynucleotide which hybridizes with an RNA encoding the senescence-induced eIF-5A polynucleotide of the plant, and

(B) regulatory sequences operatively linked to the antisense polynucleotide such that the antisense polynucleotide is transcribed; and

(2) growing said plant, whereby said antisense polynucleotide is transcribed and binds to said RNA encoding the senescence-induced eIF-5A whereby expression of the senescence-induced eIF-5A polynucleotide is inhibited.